NEW CLAIMS

New claims 9-25 have been added. No new matter has been introduced, and support for the various new claims may be found, *inter alia*, as follows.

Claims 9 – 21, and 25 are drawn to methods for detecting the presence of phenol and various substituted phenols in a test sample, utilizing bacteria engineered to express sensor-domain mutants of the DmpR protein, which confer an enhanced transcriptional activation response to phenol or substituted phenol(s), wherein the expression of a reporter gene under the control of a promoter inducible by the mutant DmpR therein provides an indication of the presence of phenol or a substituted phenol. Each of these claims is fully supported by the specification as a whole, and particularly at: page 1, lines 5-8; page 3, lines 23-28; pages 4, line 26 – page 5, line 17; page 5, line 19 – page 7, line 8; and the Examples on pages 7-12, particularly pages 11 and 12, and Figures 2-7, wherein the results of detection assays utilizing a number of exemplified DmpR mutants are presented.

Claim 22 is drawn to polynucleotides having the nucleotide coding sequences of various specifically exemplified DmpR mutants. Support for this claim may be found, *inter alia*, in the sequence listings provided for these DmpR mutants, each of which demonstrated utility in the phenol and substituted phenol detection assays described in the Examples. Similarly, claim 23, drawn to vectors containing the DmpR mutant sequences of claim 22, and claim 24, directed to host cells containing such vectors, are supported, *inter alia*, by the Examples.

REJECTIONS UNDER 35 USC 112, SECOND PARAGRAPH

Claim 1 was rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Firstly, the Examiner notes that the claims are indefinite in the recitation of the term "response", since one of skill in the art would recognize that

bacteria can respond to phenols in a variety of ways. Claim 1 has been amended to clarify that the response is the enhanced transcriptional activation of a reporter gene under the control of a promoter regulated by a DmpR protein, consistent with the Examiner's suggestion.

Secondly, the Examiner notes the lack of antecedent basis for "the bacterial DNA encoding the regulatory protein" and the indefinite recitation of the term "bacterial DNA". Claim 1 has been amended to remove the term "bacterial", thereby clarifying that the sensor domain is removed from the DNA encoding the DmpR protein. Applicants are grateful to the Examiner for having noted the above issues and request reconsideration and withdrawal of the rejection.

REJECTION UNDER 35 USC 112, FIRST PARAGRAPH

Claim 1 was further rejected under 35 USC 112, first paragraph, on the grounds that the specification does not reasonably provide enablement for a method of enhancing *any* response of *P. pudita, Acinetobacter, and E. coli* bacteria to phenols or substituted phenols by mutating the sensor domain that activates expression of *all* genes encoding *any* metabolic enzyme by mutagenic PCR or gene shuffling. The Examiner acknowledges that the specification does enable a method of enhancing reporter gene expression in *P. pudita, Acinetobacter, and E. coli* bacteria in response to phenols and substituted phenols by mutating the sensor domain of the respective recited regulatory protein that activates reporter gene expression by mutagenic PCR or gene shuffling. Claim 1 as here amended is drawn to subject matter acknowledged as being enabled by the Examiner. Applicants therefore request reconsideration and withdrawal of this rejection.

REJECTION UNDER 35 USC 103

Claim 1 was rejected under 35 USC 103(a) as being unpatentable over Pavel et al. in view of Willardson et al., Minishull et al., and Caldwell et al.

The Examiner argues that *"[o]ne would have been motivated to mutate only the sensor domain of the regulator proteins of claim 1 because of the teachings of Pavel et al. who taught that the binding specificity of regulatory proteins can be broadened by mutating only the sensor domain and not the DNA binding and transcriptional activation domains."* Applicants respectfully disagree with this reasoning, as it is clear that the chemical mutagenesis method employed by Pavel et al. was not specifically targeted to mutating *only* the sensor domain, and as the objective of the underlying study had nothing to do with the detection of organic pollutants.

Pavel et al. was not concerned with the generation of mutant regulator proteins for use in assays to detect the presence of phenolic and related organic pollutants. In this regard, the principal conclusion stated in Pavel et al. is that *"the aromatic effector activation of wild-type DmpR by the para-substituted methylphenols is a major factor limiting the catabolism of these compounds."* Given the clear purpose of the investigation reported in Pavel et al. (i.e., studying the limitations of methylphenol catabolism), it is not surprising that these authors made no statement whatsoever indicating or suggesting that *targeted* mutations to the DmpR *sensor domain* would be an important consideration in the context of phenol detection assays utilizing bacteria carrying such mutant proteins.

Moreover, Pavel et al. make no statement indicating any awareness of the problems associated with using mutant DmpR proteins in phenolic detection assays where the mutagenesis technique used to generate the mutant DmpR is predisposed to indiscriminately introduce multiple mutations outside of the sensor domain. Indeed, applicants have found no statement in any of the three secondary references indicating such an awareness or related concern. Thus, one of ordinary skill would not have gleaned from Pavel that one should take this matter into account, and, in the context of the particular application addressed by the instant invention, consider using a targeted mutagenesis approach. Applicants' method of generating DmpR mutants is specifically directed to phenolic detection assay applications (i.e., the method is one designed to

generate sensor domain-specific mutations while essentially eliminating the possibility of generating mutations outside of the sensor domain).

The invention is further distinguished from Pavel et al. inasmuch as the phenolics detected by Pavel's DmpR-E135K mutant (other than simple phenol) are not those listed as priority pollutants by the Environmental Protection Agency (Federal Register. 1998. *National recommended water quality criteria*. Fed Regist. **63**:67547-67558), whereas the DmpR mutants described in the specification do detect the seven phenolics listed as priority pollutants by the U.S. Environmental Protection Agency.

With respect to the newly added claims directed to detection assays for the recited phenolics using DmpR mutants, Pavel provides no evidence that such mutants can be created at all, let alone mutants having 20 to 60-fold enhanced effector recognition. Pavel shows a single mutant DmpR having slightly enhanced recognition of 4-methylphenol and 3,4-dimethylphenol. Pavel's DmpR mutant showed no enhancement in the recognition of phenol, whereas the method of the invention generated a mutant having a 4.7-fold enhancement in phenol recognition.

Thus, there is no clear suggestion in Pavel et al. to mutate only the sensor domain. Without any suggestion, stated or otherwise, of the need for or desirability of a targeted mutation approach, one of ordinary skill in the art would not have been led to combine Pavel with Cadwell et al., a PCR manual which describes the mutagenic PCR technique modified by applicants to generate the several DmpR mutants described in the specification.

With respect to an expectation of success, applicants cannot identify anything in Pavel et al. that would lead one of ordinary skill in the art to adopt the view that high level enhanced recognition of any particular phenol effector could be achieved using the method described in that publication, let alone the particular method taught by the present invention. Combining Pavel with Cadwell does not reach the legal threshold of

a reasonable expectation of success either, since the combination still does not provide any indication or suggestion that the *applicants'* method would indeed result in DmpR mutants having such high-level effector recognition properties. As noted by the Examiner in an earlier Office Action, there is a distinct lack of predictability in the art of modifying protein structures in the context of functional biological properties.

Combining the above-two references with Willardson would appear to add very little if anything as well, because Willardson relates to the use of a *wild-type* regulator-reporter, not sensor domain regulator *mutants*. The statement referenced by the Examiner (*"the development of this biosensor for toluene and its derivative compounds demonstrates the feasibility for constructing similar biosensors with specificity for other organic contaminants by using their corresponding transcriptional activators"*) was clearly directed to the use of *wild-type* transcriptional activators, not *mutations* thereof. Thus, Willardson also does not contain any suggestion or motivation to generate DmpR sensor-domain mutants (or related regulator protein sensor-domain mutants) having enhanced effector recognition functions and, thus, particular utility in the identification of effector organics not recognized by wild type systems. Certainly, there is no teaching in Willardson that could provide one with a reasonable expectation of success in making the invention claimed here.

Lastly, applicants do not agree that Minshull could have assisted one of ordinary skill, since Minshull only mentions the general idea of altering the specificity of biosensors for detecting different chemicals. As the Examiner would undoubtedly agree, there is no motivation in Minshull for the generation of DmpR mutants particularly, or any indication of the need for or desirability of sensor-domain-targeted mutations in the context of phenol detection assays. Additionally, Minshull describes a fundamentally different technical approach involving cycled recombination between related genes to achieve diversity. In this regard, Minshull's "recursive sequence recombination" is not in any way similar to PCR mutagenesis. Recursive sequence recombination will diversify DNA in ways that are not limited to single base substitutions (as in PCR mutagenesis).

Minshull's method would be more appropriate if the aim were to change effector specifically to something significantly different from the natural effectors, or possibly to combine traits of two different regulatory proteins.

The aim of what the applicants in this case pursued was the creation of slightly modified sensor domain protein structures capable of recognizing and complexing with variants of the natural effector of the wild-type sensor (i.e., substituted phenols). This aim may not have been achieved utilizing a recursive sequence recombination approach, and in any event, no suggestion that a mutational approach aimed at single base substitutions can be found in Minshull. Indeed, in this respect, Minshull may well have led one away from the path adopted by applicants. Furthermore, none of the cited references contain an indication that mutational techniques which principally introduce single base substitutions (like PCR mutagenesis), and *only* in the DmpR sensor domain, are desirable or preferred for the particular application to which this invention is directed.

The Examiner refers to Minshull as providing an example of mutating only a specific catalytic domain of a polyketide synthetase to optimize only that desired catalytic activity, concluding that, to one of ordinary skill, this information would have rendered the mutation of the DmpR sensor domain obvious. Applicants do not see such an example in the Minshull patent, and therefore request clarification. Nevertheless, it must be recognized that the present invention is directed to the mutation of an organic effector sensor domain, not a catalytic domain, for the express purpose of developing assays for the detection of organic pollutants. Knowledge concerning protein structure and effector interaction is still very basic. Furthermore, applicants can think of no reason why the catalytic domain of a polyketide synthetase (the active portion of an enzyme) should be considered informative as to interaction between an effector and the sensor domain (non-catalytic) of a regulatory protein (not an enzyme). They are very different types of proteins.

Thus, there is no clear suggestion in Minshull for the targeted mutation of a sensor domain in DmpR or related proteins for the express purpose of expanding or enhancing DmpR-effector recognition, with the further express purpose of utilizing such mutations in a biological assay for detecting phenol pollutants. Certainly nothing in Minshull could lead one of ordinary skill in the art to have any expectation of being able to successfully generate DmpR sensor-domain mutants having high level enhancement and/or expanded effector recognition using the targeted PCR mutagenesis approach adopted by applicants.

In conclusion, applicants contend that these references likely would not have been combined by one of ordinary skill in the art at the time the subject invention was developed. Even if one were to combine the four cited references without the benefit of the invention, the combination would not have suggested the use of a *targeted* mutational approach or the use of a technique principally directed to single base substitutions, and would not have led one to reasonably expect that mutations effective for the purpose central to the invention could be generated with the degree of success reflected by the results presented in the specification (and summarized in the table above).

In view of the above remarks, applicants respectfully invite the Examiner to agree that the cited references would not have suggested the invention or provided a reasonable expectation of success. Applicants also trust that the reasoning provided herein will apply equally in relation to the newly added claims, all of which contain limitations clearly not suggested by the cited references, nor predictable in view of the cited references or the prior art as a whole. Accordingly, applicants request reconsideration and withdrawal of the above rejection, as well as consideration of these remarks in relation to the Examiner's review of the new claims.

In view of the above amendment to claim 1, and the foregoing remarks, applicants respectfully request withdrawal of all pending rejections and an indication of allowability of claim 1 and new claims 9-25. If in any respect the Examiner is not persuaded, applicants request a telephonic interview prior to further written action.

Respectfully submitted,

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